

Supplementary Material

Scandium(III) Triflate as a Lewis Acid Catalyst of Oxime Ligation

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Experimental Details

Materials and Methods: All solvents and chemicals were purchased from commercial sources and used without further purification: DMF (dimethylformamide), CH₃CN (acetonitrile), CH₂Cl₂ (dichloromethane) and (C₂H₅)₂O (diethyl ether) from Fisher, TFA (trifluoroacetic acid), and DIEA (*N,N*-diisopropylethylamine) from Sigma-Aldrich, 4-Methylpiperidine and anisole from Alfa Aesar, and HCTU (*o*-(1-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) from Peptides International. Fmoc-protected and Boc-protected amino acids were purchased from Bachem. Fmoc Rink Amide polystyrene resin was purchased from Novabiochem. PAM-Ser-Boc preloaded resin was purchased from Peptides International. Acetylbenzoic acid, *N*-hydroxy-succinimide, aniline and the three regioisomers of phenylenediamine were purchased from Sigma Aldrich. Scandium(III) triflate was purchased from Strem Chemical. Water was purified using a Millipore Milli-Q purification system.

Fmoc-SPPS: Peptides were chain assembled on Rink Amide polystyrene resin by manual couplings. All peptides were synthesized at 0.2 mmol scale of resin. Amino acid couplings were carried out with the equivalent ratio of [5]:[5]:[7.5] of [Fmoc-protected amino acid]:[0.4 M HATU in DMF]:[DIPEA] for 20 minutes following 20% 4-Me-piperidine (2 × 2 min) treatments for *N*-Fmoc deprotection. Peptides were cleaved from the resin using a standard TFA cleavage cocktail (95% TFA, 2.5% TIPS, 2.5% H₂O) for 2 hrs, filtered, precipitated with cold diethyl ether and lyophilized from buffer (0.05% TFA in water), yielding the peptide as the TFA salt.

Purification and Analysis: Peptides were purified by reversed-phase HPLC (RP-HPLC). Analytical RP-HPLC was carried out on an Agilent 1100 Series HPLC on a Phenomenex Jupiter Proteo column (4 μm, 90 Å, 150 × 4.6 mm) at a flow rate of 1 mL/min. Analytical injections were monitored at 214 nm. Preparative RP-HPLC was performed on a Waters Delta Prep 4000 equipped

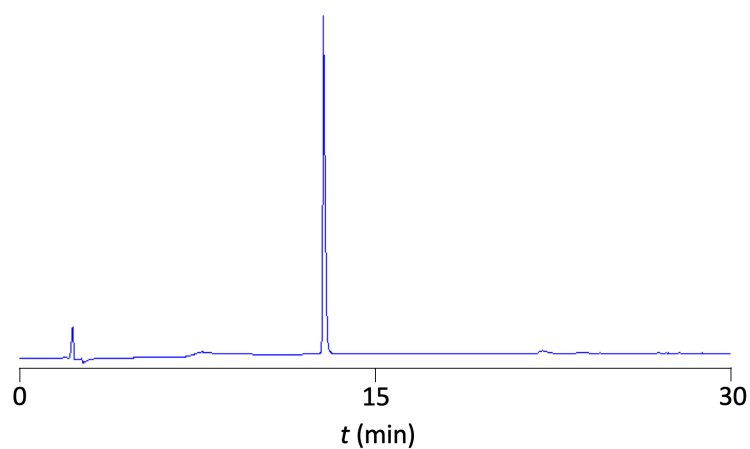
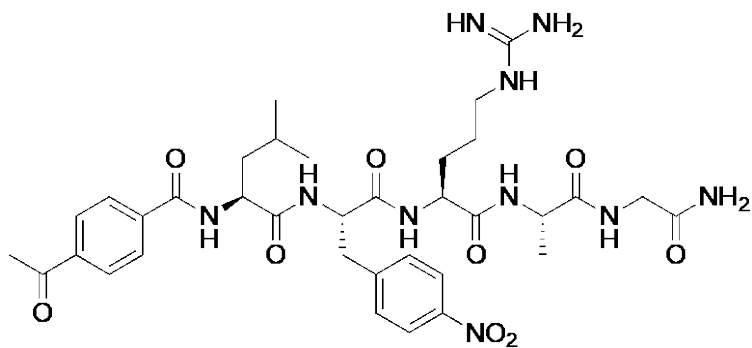
with a Waters UV detector model 486 and a Phenomenex Jupiter Proteo column (10 μm , 90 \AA , 250 \times 21.20 mm) at a flow rate of 15 mL/min. Preparative injections were monitored at 220 nm.

Peptides were characterized using electrospray ionization MS on a LC/MS API 2000 Plus triple quadrupole mass spectrometer (Sciex). Peptides masses were calculated from the experimental mass to charge (m/z) ratios from all of the observed protonation states of a peptide by using the onboard analyst software package (Sciex).

Buffer A: H₂O (0.05% TFA).

Buffer B: MeCN/H₂O (9:1) (0.05% TFA).

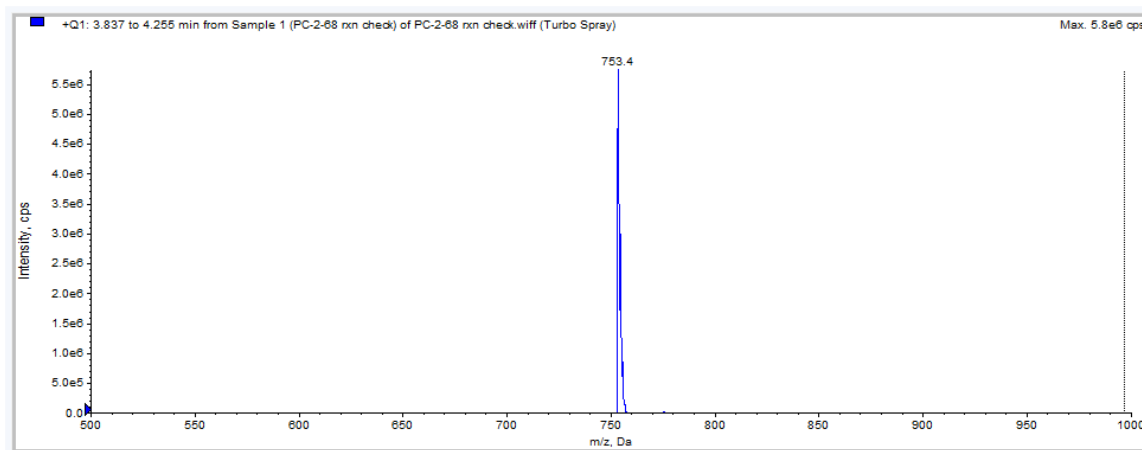
Analytical Data for Peptide AcBza-LY(NO₂)RAG:



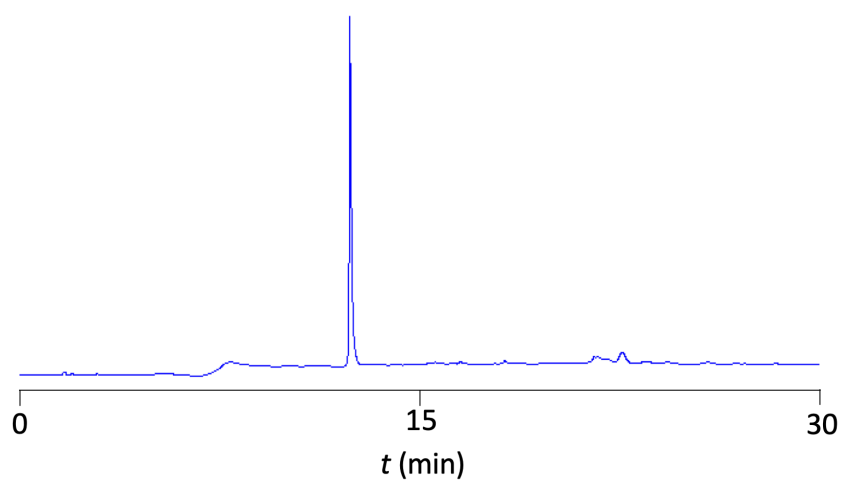
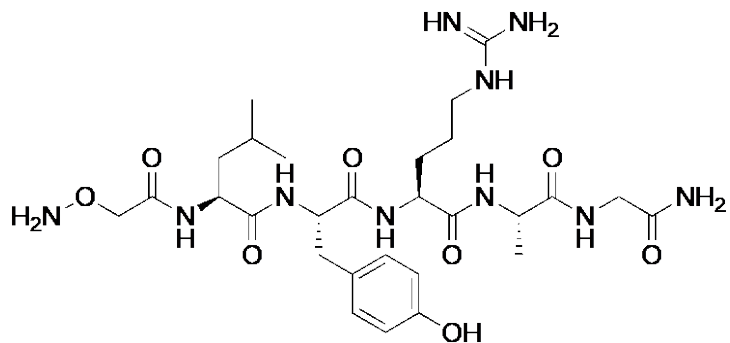
Exact Mass: 752.36

Expected M+1 Mass: 753.37

Found M+1 Mass: 753.4



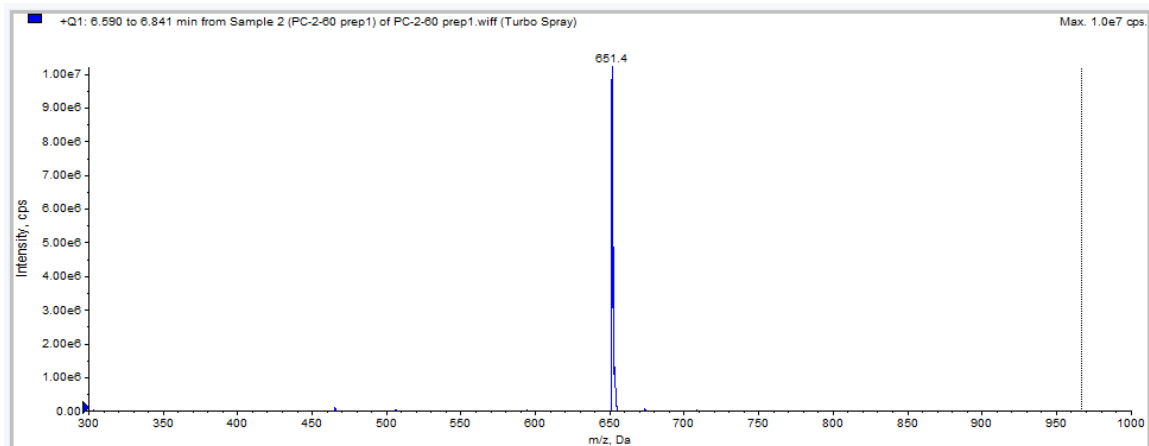
Analytical Data for Peptide Aoa-LYRAG:



Exact Mass: 650.35

Expected M+1 Mass: 651.36

Found M+1 Mass: 651.4



Reaction Setup for Oxime Ligation Reactions:

To a 1.5 mL Eppendorf tube there was added lyophilized peptide(s) and/or tag molecules in the required quantities/molarities as described in **Figure 2**, followed by 500 μL of 100 mM MES buffer, pH 6.8, which also contained the desired nucleophilic catalyst at the proper concentration, if required. For reactions with scandium(III) triflate, the dry material was added to the tube prior to the addition of the buffer. The reaction was adjusted to the desired pH using aqueous 10% HCl solution. The reaction mixture was then heated to 25 $^{\circ}\text{C}$ and 10 μL aliquots were taken at the desired time points, filtered through a PTFE syringe filter, and diluted to a volume of ~ 400 μL which were then analyzed by RP-HPLC and MS-TOF.